## PLANT RESPONSES TO THE ENVIRONMENT

Panel Manager - Dr. Peter B. Goldsbrough, Purdue University
Program Director - Dr. Gail McLean

Awards in this area support research aimed at understanding the plant's response to environmental factors, both natural and anthropogenic. The major goal of the program is to provide the basic knowledge needed to devise strategies for decreasing the impact of environmental stress and for adapting agricultural and forest practices to possible changes predicted to accompany global climate fluctuations.

Studies on mechanisms at the whole plant, cellular, or molecular level which explain organismal response are emphasized. The environmental factors of interest include water, temperature, light (including UV-B), nutrient, and atmospheric chemical composition (including carbon dioxide and other greenhouse gases, sulfur dioxide and ozone).

### 2000-00664 Molecular Genetic Analysis of Low Temperature Signal Transduction in Plants

Zhu, J.K.
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Grant 00-35100-9426; $250,000; 3 Years

Low temperature is one of the most common environmental factors influencing plant growth and crop productivity. The long-term goal of this research is to understand how cold environments are perceived by plants and to use this knowledge to improve plant tolerance to chilling and freezing conditions. We have identified several Arabidopsis plant mutants that are defective in cold signal transduction. Detailed genetic, molecular, biochemical, and physiological characterization of the mutants will be conducted to help understand how plants respond to cold environments. One of the mutated genes will be cloned. The findings will significantly advance our understanding of biological sensing of low temperature cues and enhance our ability to develop rational strategies for engineering chilling and freezing tolerant crops.

### 2000-00647 Chilling Tolerance in Wild and Cultivated Tomatoes

Bloom, A. J.; St. Clair, D. A.
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Grant 00-35100-9530; $180,000; 3 Years

In a cultivated tomato (*Lycopersicon esculentum*), a brief exposure of the roots to chilling temperatures damaged root ammonium absorption and caused the shoots to wilt. Under the same treatment, a wild relative from high altitudes (*Lycopersicon hirsutum*) showed little change in ammonium absorption and the shoots did not wilt. In offspring from crosses between the two species, the chromosomal locations associated with these traits were identified. Ammonium absorption was associated with a location on chromosome 3. Shoot wilting after two hours at 4ºC was highly correlated with a location on chromosome 9. Recovery from wilting after six hours at 4ºC was associated with a location on chromosome 7. The proposed research will further characterize the genetics and physiology of these traits. Additional crosses between the hybrid offspring and the cultivated tomato will be made. Genetic markers will allow identification of

individual plants in which the genetic material derives from the cultivated tomato except for the specific chromosomal locations associated with ammonium absorption or shoot wilting. Physiological studies will examine these individuals to determine the role of ammonium absorption and water movement in the overall chilling response of tomato and the relationship among root ion absorption, water movement, and growth. This project involves a collaborative effort between a geneticist and a physiologist. Our results will provide insights about the genetic bases of plant chilling damage, nutrient absorption, and water movement.

**2000-00676 C BUDGET, Sap Sugars, Root Anatomy: $O_3$ Effects in Contrasting Phloem Loaders**

Grantz, D.A.
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Grant 00-35100-9181; $210,000; 3 Years

Ozone is the most damaging air pollutant to crops and vegetation. Ozone inhibits photosynthesis and export of sugars from leaves to roots and fruit. Our studies with Pima cotton indicate that sugar movement is inhibited more than photosynthesis. Our previous research funded by USDA showed that ozone alters the yield and root development of Pima cotton and the ratio of key sugars in root tissue. The current study uses muskmelon and Pima cotton to ask whether the contrasting sugar transport used by cotton and muskmelon can be manipulated to demonstrate the mechanism of ozone effects. We will extract sugars from leaf and root tissue and use aphids to sample the contents of the phloem which moves sugar from leaves to roots. We will measure root carbon budgets to determine the importance of sugar export and changes in root respiration rates. We will characterize the altered shape, size and internal anatomy of the root system with emphasis on water transporting tissues in the roots. The results of the study will integrate at the whole plant level the effects of ozone. The results also will suggest management and breeding objectives to improve plant resistance to ozone.

**2000-00659 High Light Acclimation: Genetic and Molecular Analysis Im, C.**

Carnegie Institution of Washington; Department of Plant Biology; Stanford, CA 94305

Postdoctoral Fellowship; Grant 2001-35100-10108; $85,000; 2 Years

Light is essential to photosynthetic organisms, since it provides energy that fuels the fixation of inorganic carbon through photosynthesis. However, light has the potential to be toxic when photosynthetic organisms are exposed to high light that exceeds the capacity of photosynthesis. Photosynthetic organisms must modulate their photosynthetic activities, eliminate excess absorbed light energy in a harmless manner. Despite numerous biochemical studies focused on how plants acclimate to high light, little is known about changes in and the control of gene expression during high light stress. Modulation of the activities of specific genes can help cells adjust their metabolic processes to a constantly changing environment. Identifying high light-activated gene (HLAG) provides ‘foundational’ information that will help establish the molecular basis of acclimation. The work that I am proposing emphasize the use of genetic and molecular technologies to obtain a global view of gene expression upon exposure of photosynthetic organisms to high light. I will take advantage of a unicellular green alga *Chlamydomonas*

*reinhardtii* since its photosynthetic apparatus is essentially identical to that of vascular plants, and it is amenable to both molecular and genetic manipulation. The major aims of the work are: (1) Isolate HLAGs using differential display technique, (2) investigate the involvement of photoreceptor(s) in activation of HLAG, (3) analyze the expression of HLAG under different stress condition, (4) investigate the sub-cellular localization of proteins encoded by HLAG, (5) generate strains that under- or over-express HLAG to determine their function(s) in photoprotective processes, and (6) identify specific regulatory elements that control the expression of HLAG.

**2000-00735 Mechanisms of Rapid Acclimation to Low Oxygen Stress in Maize Root Tips**
Roberts, J.K.M.
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Grant 00-35100-9166; $250,000, 3 Years

Heavy rain or irrigation, combined with inadequate drainage of water from soil, prevents adequate aeration of crop roots, and this low oxygen stress can depress crop yields. When root tips of intact corn plants are exposed to mild low oxygen stress, within a couple of hours they are able to improve their tolerance of severe low oxygen stress. This dramatic adaptive response depends on protein synthesis, and large numbers of proteins are synthesized during this response. The aim of this project is to identify proteins that contribute to adaptation, using the techniques of protein biochemistry and mass spectrometry. We will focus on proteins involved in important aspects of cell function, including energy metabolism, intracellular pH regulation and the translation of nucleic acids into proteins. The gene products which change in abundance and phosphorylation state (coincident with rapid acclimation) will be identified to give an integrated view of plant responses to stress. This new approach will allow us to select, from the many molecular changes triggered by low oxygen stress, an important subset that is associated with improved plant performance. This project will also develop and test new methodologies to detect and identify proteins involved in the low oxygen stress response, which will also be of use to other scientists studying other plant systems and environmental responses.

**2000-00660 Interactions Between Root Growth Zones and the Surrounding Soil**
Silk, W.K.
University of California, Davis; Department of Land, Air, and Water Resources; Davis CA 95616-8627
Grant 00-35100-9531; $150,000; 3 Years

The pH of the soil next to the root has a large effect on rates of uptake of both beneficial nutrients and toxic metals into plants. Thus soil acidity is important in regulating the rate of introduction of mineral elements into the food chain. This study will examine the ability of the root tip to change the pH of its immediate environment and will characterize the rhizosphere, *i.e.* the zone of influence of the root in the neighboring soil. Miniprobes will be used to measure effects of bulk soil properties, water stress, and temperature on pH of the root surface and the rhizosphere. These laboratory results will be used to test and extend a recently formulated mathematical model to predict the acidity around growing root parts. Then plant species with differing capacities for rhizosphere

acidification will be tested for improved uptake of mineral nutrients, particularly phosphate. Effects of acidification potential on mineral nutrition will be tested by applying nutrients in a defined soil layer, allowing a particular root location to grow past the nutrient–rich layer, assaying the spatial distribution of the mineral content, and using growth analysis with the nutrient density data to calculate local nutrient deposition rates within the growth zone.

**2000-00715 Protein Phosphorylation and Xanthophyll Cycle Dynamics Dependent on Lifeform**

Demmig-Adams, B.; Adams III, W.W.
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Grant 00-35100-9564; $150,000; 3 Years

Evergreen species preserve green leaves throughout periods of extreme environmental stress while many mesophytic crop species do not. The purpose of this project is to investigate the involvement of a special form of photoprotection in the chloroplasts of highly stress-resistant evergreen species. It is hoped that this information will assist in the development of more stress-resistant crops. The special form of photoprotection induced by environmental stresses involves a continuous and highly efficient dissipation of excess absorbed light that can potentially kill the leaves. This project will examine whether energy dissipation becomes "locked-in" under stress via modification of structure and function of specific photosynthetic membrane proteins by protein phosphorylation. Goals of this project include identification of contrasting patterns of protein phosphorylation and energy dissipation between evergreens and crop species and identification of the proteins involved. Mechanistic features of sustained energy dissipation will be assessed through inhibitor treatments.

**2000-00997 The Reproductive Biology of Hyperaccumulator Plants**

McKenna, M.A.
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Sabbatical Grant; Grant 00-35106-9533; $64,173; 1 Year

This research examines the role of nickel in the reproductive biology of *Alyssum murale*. *A. murale* is a nickel hyperaccumulator; it has an unusual ability to accumulate nickel from the soil to reach very high levels (2%) in shoot tissue. Although plants require small quantities of nickel, high levels of nickel are toxic to most plants. Research in Rufus Chaney's laboratory at USDA BARC has explored the use of *Alyssum to* absorb nickel from industrial contaminated sites or from mineralized soils (phytoextraction). *Alyssum* biomass can be burned to produce energy and nickel can be recovered from plant ash. Seed set is high when *A. murale* is grown on nickel-rich soil, but when *A. murale* was grown on a coastal plain soil with low nickel content, no seed formed on flowering plants. In this study, seed set and seed quality from controlled crosses will be compared in *A. murale* plants grown on coastal plain soil with varied nickel content. Nickel effects on pollen germination and growth will be studied using *in vitro* pollen growth techniques. The nickel content of floral and vegetative tissues will be measured using inductively coupled plasma spectrometry or atomic absorption spectrometry. Urease activity will also be examined, since nickel acts as a cofactor for this enzyme in higher plants. This research will further our understanding of the biology of hyperaccumulator plants and aid in the development of *A. murale* for phytoextraction.

**2000-00655 A Genetic Approach to Determine the Role of GAD2 in the Plant Stress Response**

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Strengthening Award; Grant 2001-35100-09930; $150,000; 3 Years

The non-protein amino acid, gamma-aminobutyric acid (GABA), rapidly accumulates in plants upon exposure to stress. In plants, GABA accumulation is controlled by the enzyme glutamate decarboxylase (GAD). Plant GADs differ from bacterial and mammalian GADs because they have a calmodulin-binding domain (CaM-BD). Furthermore, GAD activity is stimulated by $Ca^{2+}$/calmodulin (CaM) indicating that plant GADs may be part of a $Ca^{2+}$/CaM-mediated signa1 transduction pathway. This hypothesis is supported by a series of recent findings that demonstrate (i) GABA alters plant growth and development (ii) GABA activates enzymes and induces gene expression, and (iii) plants have GABA or GABA-like receptors. We plan to use a series of *Arabidopsis* mutants that have altered amounts of the major form of GAD, called GAD2, to determine the role of GAD and GABA in the plant stress response. We plan to use the genetically altered plants to determine the GAD/GABA signal transduction pathway in plants. Since GAD and GABA may play key roles in plant signaling, especially in response to biotic or abiotic stress, it is important to understand the environmental and cellular factors that regulate GAD gene expression, GAD enzyme activity, and GABA levels in plants so that new environmentally safe and inexpensive agricultural strategies can be developed. These strategies could include the use of foliar sprays or genetically engineered plants that alter agronomically important baits such as plant height, resistance to certain insects, increased crop productivity and tolerance to environmental stresses.

**2000-00687 Functional Analysis of the Stress 70 Chaperone Family in *Arabidopsis***

Guy, C.
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Grant 00-35100-9532; $260,000; 3 Years

Much of the nation's major crop growing regions are subject to temperature extremes that cause various forms of heat or cold stress. Economic losses to the aggregate of all crop commodities from temperature stress in the last 10 years could be conservatively estimated to be in the billions of dollars range. Plant cells possess a specialized group of proteins whose function it is to help other types of proteins fold and assemble into a biochemically or physiologically functional structure. Such helper proteins are known as molecular chaperones. One class of these helper proteins includes the heat shock 70 protein made when plants are exposed to a sudden rise in temperature. The purpose of heat shock 70 protein, and other heat shock proteins, is to protect proteins of the cell from heat damage or repair them. The objective of this project is to develop information that will lead to a better understanding of the function of the stress 70 molecular chaperones in plants under stressful as well as non-stressful conditions. The primary objective of this project is to describe some of the key biochemical mechanisms that underlie the physiological functions of the stress 70 family of chaperones in plants. This effort will take advantage of the power of over- and under-expression of transgenes and "knock-out" mutants of the stress 70 molecular chaperones in plants to unravel some of the whole

organism biological processes relying upon, or requiring, involvement and proper function of the stress 70 chaperones.

**2000-00729 Physiological Restriction to Cotton Leaf and Canopy Photosynthesis under Drought Stress**

Earl, H.J.
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New Investigator; Grant 00-35100-9404; $100,000; 2 Years

Drought stress constitutes the single greatest limitation to productivity of field crops in the southeastern United States. Modern plant breeding techniques have the potential to produce more drought tolerant crop varieties, but these efforts would benefit from an improved understanding of the physiological effects of drought on crop growth. One of the primary effects that drought stress has on crops is to limit the rate at which carbon from atmospheric $CO_2$ is assimilated into new plant biomass via the process of photosynthesis. This occurs by several distinct mechanisms. At the level of individual leaves, drought stress induces closure of stomata (pores that regulate movement of gases in and out of leaves), to reduce transpirational water losses. However, stomatal closure also decreases inward diffusion of $CO_2$, causing leaf internal $CO_2$ to become limiting to photosynthesis. Drought stress may also induce non-stomatal limitations; that is, the intrinsic ability of leaves to utilize available leaf internal $CO_2$ may be compromised. In the present work, a novel technique is used to impose drought stress events of varying intensity and duration on greenhouse-grown cotton plants. Stomatal and specific non-stomatal limitations to photosynthesis are then quantified during the stress and following rewatering, using an approach that combines gas exchange and chlorophyll fluorescence measurements. In a second study, these techniques are used to investigate the occurrence and magnitude of non-stomatal limitations to photosynthesis of cotton during drought stress and upon recovery from drought stress under field conditions.

**2000-00665 Stress Response of Fermentative and Glycolytic Genes in *Arabidopsis***

Shih, M.C.
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Grant 99-35100-7597; $150,000; 3 Years

Most crop plants, including barley, maize, sorghum, and wheat, can only tolerate very transient flooding. In contrast, rice plants can survive much longer under flooding conditions. How this differential flooding tolerance is achieved remains largely unknown. We have been studying the effects of flooding-induced hypoxia (low oxygen) on the expression of fermentative and glycolytic genes in *Arabidopsis thaliana.* We found that the induction of these genes is essential for the survival of *Arabidopsis* plants during hypoxia. We have isolated a class of mutants, designated as *ear*, that are defective in regulating the expression of the gene that encodes alcohol dehydrogenase *(ADH)* in *Arabidopsis.* Analyses of some of these mutants indicated that multiple signaling events mediate hypoxic induction of *ADH* and other glycolytic genes.

Two major aims are proposed in this proposal: (1) to investigate how a decrease in the overall induction of glycolytic and fermentative enzymes affects the survival of plants during flooding; and (2) to examine whether glycolytic and fermentative genes are coordinately regulated during flooding. To achieve these goals, the following experiments

are proposed. (i) Genetic studies will be performed to determine the total number of *ear* genes. (ii) Cloning and characterization of the *AAR* gene will be attempted. (iii) Temporal expression of glycolytic and fermentative genes during hypoxia in wild-type and *ear* mutants will be examined. Results from the proposed studies should allow us to identify key components in hypoxia signaling pathways. This information is essential in our efforts in developing crop plants with improved flooding tolerance.

**2000-00722 A Novel Source of Cold-tolerant $C_4$ Photosynthesis for Maize**

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Grant 00-35100-9057; $130,000; 2 Years

Some of our most important agronomic species (*e.g.*, corn, sorghum, sugar cane, switchgrass) are extremely efficient producers when grown at high temperatures, but both early season growth and the extent of their growing range are limited by poorer performance at low temperatures. Such crops are extremely productive because they use a specialized photosynthetic pathway called C4 photosynthesis, which has the highest efficiency of photosynthesis known. This efficiency is lost at temperatures below 20°C. The grass *Miscanthus x giganteus* is from the same taxonomic group as sugar cane, sorghum and corn and uses the same C4 photosynthetic pathway. However, in contrast to these other species, it performs efficient photosynthesis at temperatures as low as 5°C. Thus, this species has solved the problem of C4 photosynthesis at low temperatures. Preliminary investigations have suggested a potential mechanism for cold tolerance in *Miscanthus*: One particular enzyme of the C4 pathway (pyruvate orthophosphate dikinase, PPDK) functions efficiently at low temperatures. We aim to determine the physiological basis for effective low-temperature C4 photosynthesis in *Miscanthus* and to identify the genetic components responsible. We will accomplish this by comparing C4 photosynthesis in corn and *Miscanthus* under optimal and low temperatures, comparing gene sequences encoding the proteins of the photosynthetic machinery from corn and *Miscanthus*, and examining how they differ from corn. This will lead to greater understanding of the mechanisms for cold-tolerant C4 photosynthesis and will provide a novel source of genes for improving cold tolerance in corn, sorghum, sugar cane and switchgrass.

**2000-00716 Molecular and Genetic Analysis of Phosphate Starvation Response**

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Grant 00-35100-9370; $180,000; 3 Years

Phosphorus is one of the major macronutrients required by plants. Plants have developed many adaptive mechanisms to overcome the deficiency of phosphate (Pi). These mechanisms involve highly coordinated expression of genes. One of the important questions that still needs to be answered is how plants sense changes in the levels of Pi and transmit this information to elicit a response. In this proposed study, we plan to analyze the signal transduction during phosphate starvation. In order to accomplish this goal, transgenic plants expressing reporter genes such as luciferase and gus under the

regulation of Pi starvation induced gene *(AtPT2)* promoter will be mutagenized. Some of the mutants with altered expression of the reporter genes should represent impaired signaling pathway leading to aberrant gene expression. Further characterization of these mutants will lead to isolation of genes involved in phosphate starvation response mechanism. This knowledge will help identify key molecular determinants required for genetic modification of plants to improve Pi efficiency. Another major research objective is to characterize the recently identified phosphate starvation induced phosphatase gene. Phosphatases are one of the important components of Pi starvation response mechanism in plants. A better understanding of the regulation of phosphatase gene will aid in developing strategies to genetically modify plants to enhance Pi availability. Phosphorus efficient plants should help farmers maintain high crop productivity while reducing the production cost and the impact of production on the environment.

**2000-00656 Molecular Analysis of Oxidative Stress Signaling in Plants**
Sheen, J.
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Grant 00-35100-9345; $210,000; 3 Years
Plants respond to many abiotic and biotic stresses through accelerated generation and accumulation of reactive oxygen species, including hydrogen peroxide ($H_2O_2$). Despite the recognition of $H_2O_2$ as a central signaling molecule in stress and wounding responses, pathogen defense, regulation of cell cycle and cell death, little is known about how the oxidative signal is received and transmitted in plant cells. The long-term goal of this research is to unravel the molecular mechanisms underlying oxidative stress signal transduction leading to cellular responses in plants. We have identified the first $H_2O_2$-activated plant protein kinase, ANP1, utilizing an Arabidopsis protoplast transient expression system. Preliminary studies with a tobacco ANP1 homologue, NPK1, in transgenic tobacco also suggest the importance of oxidative stress signaling in the establishment of multiple stress tolerance. We propose to use a combination of approaches to elucidate the functions of ANP1 in a model plant *Arabidopsis thaliana* and identify other key molecular regulators of the ANP1 signaling cascade. To elucidate the ANP1 function *in planta*, *Arabidopsis* with altered ANP1 activities will be used to examine gene expression programs, define target genes, and determine developmental processes and stress responses affected by the ANP1 signaling cascade. The analysis of the ANP1-mediated pathway will enhance our understanding of oxidative stress signaling and might define new potential regulators of multiple stress and pathogen tolerance in plants. Since the ANP1 signaling cascade seems to be conserved in monocot and dicot plants, our study of the ANP1 pathway will have significant applications in agriculturally important plants.

**2000-00677 The Contribution of Deep Roots to Whole-Plant Water Relations and Xylem**
Jackson, R.B.; Pockman, W.T.
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Grant 00-35100-9127; $135,000; 2 Years 1 Month

The goal of this proposal is to understand the contribution of deep roots for the functioning of plants. We propose that deep roots differ fundamentally from shallow roots and stems in their anatomy and in their ability to transport water. Such differences are important for the many rangelands in the U.S. that are being invaded by deep-rooted shrubs and trees. They are also important for the amount of water that recharges aquifers in central Texas (the site of the proposed work) and the many other sites where people obtain drinking water from aquifers. In this project, we will use caves to study trees at different depths underground. We will study the physiology of the trees and measure how much water they take up from those depths. The research should improve our understanding of plant responses to drought. It should also help us learn why deep-rooted woody species that invade rangelands, such as juniper and mesquite, are so successful.

**2000-01143 High-Performance Liquid Chromatograph for Study of Wounding and Salinity Stress on Plant Membranes**

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Equipment Grant; Grant 2001-35106-09868; $21,191; 1 Year

Biological membranes are critically important to the health and viability of plant cells and tissues. Complex lipid molecules, such as phospholipids, glycolipids, and steryl lipids, constitute a major portion of membranes and are largely responsible for the vital functions that membranes fulfill in plants. Environmental stresses, such as wounding and salinity, profoundly influence the integrity of cell membranes. Study of wounding and salinity stress in crops is becoming increasingly important in light of the higher consumer demand for a diversity of fresh-cut (wounded) products such as vegetables, fruits, and flowers, and because of heightened environmental concerns over diminishing supplies of high quality irrigation water and the need for wastewater utilization in cropping systems. The aim of this research is to incorporate the use of a high performance liquid chromatograph for rapid and efficient laboratory analysis of membrane lipids in wounded and salinity-stressed tissues. Data will increase our understanding of cell membrane-related characteristics involved in decomposition and repair of wounded plant tissues and in salinity tolerance. Ultimately, the findings obtained from the research should aid in identifying key membrane lipid metabolic pathways, eventually leading to the practical application of modern plant genomic techniques and the ultimate goal of quality maintenance of fresh-cut products and development of plants suitable for saline agroenvironments.

**2000-00641 Influence of Environment on the Structure and Dynamics of Plant Cuticular Surfaces**

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Grant 00-35100-9462; $110,000; 2 Years

The outermost surfaces of leaves and fruits of higher plants consist of a cuticular membrane that controls the plant's interactions with the environment and acts as a first

line of defense against pathogenic attack. The cuticle can regulate the diffusion of molecules into plant tissues and prevent water loss. The chemical composition and microstructure of the cuticle's surface controls the adsorption of agriculturally important chemicals. Breakdown of this barrier contributes to an estimated $10 billion annual loss due to crop damage. Since cuticular efficacy is governed by surface and interfacial phenomena, our objective is to combine surface science approaches with methods of probing intracuticular chemical dynamics. This combination will afford a molecular level understanding of the structure-function relationships of cuticular materials and how they are modified by environmental factors such as relative humidity (water uptake), temperature and the adsorption and absorption of agrochemicals. Only with the recent introduction of surface science tools into biology, in particular atomic force microscopy and secondary-ion mass spectroscopy, have these types of molecular level surface chemical and structural studies become feasible. Here the structural and mechanical behavior at plant surfaces and interfaces, in response to agrochemical adsorption coupled with environmental factors (i.e. relative humidity and temperature), will be investigated. These studies will be complemented by chemical dynamics studies using solid-state nuclear magnetic resonance and secondary ion-mass spectroscopy to examine surface chemical composition and diffusion of chemicals through the cuticle. The ultimate goal of this work is the rational design of crop protection schemes.

**2000-00675 Aluminum Tolerance Mechanisms in Maize: The Role of Anion Channels in Organic Acid Exudation**

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Grant 00-35100-9280; $170,000; 3 Years

Aluminum (Al) phytotoxicity limits agricultural productivity on the acid soils that comprise approximately 30% of the world's total land area. Al solubilizes into acid soil solutions and accumulates to toxic levels, inhibiting root growth and function. However, plants have evolved tolerance mechanisms enabling them to grow in soil environments where the roots are exposed to potentially high levels of aluminum. Plant breeders have exploited these traits to develop A1 tolerant varieties of certain crop species. In order to improve these A1 tolerant crop species, there is a need for a better understanding of the physiological, biochemical and molecular basis for Al tolerance. Whole plant studies in maize have shown an Al-induced release of Al-binding ligands (primarily organic acids) at the root apex that provides Al-tolerance by chelating and reducing the activities of toxic Al in the rhizosphere. We have used electrophysiological techniques to identify Al-inducible proteins involved in the transport of organic anions in cells isolated from the root apex. These studies indicate that Al-inducible channel proteins play a key role in maize A1 tolerance, as the primary transport pathway for organic acid exudation. The aim of our research is to complete a comparative characterization of the physiology of Al-induced organic acid release in intact roots of the A1 tolerant and sensitive maize genotypes and to concurrently characterize the properties of the Al-activated anion channel proteins using electrophysiological techniques. The latter will allow us to compare processes at the cellular and subcellular levels with the investigations of the Al tolerance system operating in the intact root. The knowledge gained from this study

should enhance our understanding of the cellular and molecular basis of A1 tolerance mechanisms in crop plants.

**2000-00734 Mechanisms by Which Water Deficit Alters Cell Division in Developing Maize Kernels**

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Grant 00-35100-9279; $150,000; 2 Years

The goal of the proposed studies is to understand the cellular and physiological bases for altered cell division in maize plants subjected to water deficit during early reproductive development. This, in turn, will provide information needed to identify which genetic systems can be manipulated to improve the performance of grain crops during water deficit. The early reproductive stages of maize kernel development are recognized as being particularly vulnerable to drought and responsible for stress-related economic loss. Our previous work on this project indicated that cell division in the affected tissue is sensitive to stress and is inhibited by the stress hormone abscisic acid (ABA). Other studies indicate that kernel abortion is affected by tissue water and carbohydrate status. The proposed work will evaluate the postulated involvement of these and other processes by determining the mRNA levels of a broad array of gene products representing the spectrum of response pathways in plants. To test postulated production of signals generated in the post-phloem pathway of maize kernels, levels of signaling molecules will be localized and related to stress-induced loss of kernel set. The involvement of ABA in kernel stress responses will be assessed using vp1 mutant plants, which are defective for ABA signaling. The new knowledge from this project will help lead to increased stability of yield performance with respect to unpredictable weather patterns and thereby contribute to a sustainable agriculture.

**2000-00712 Ozone and Guard Cell Function: Mechanisms of Action and Whole Plant Impacts**

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Grant 00-35100-9420; $170,000; 3 Years

The air pollutant ozone (03) is generated when nitrogen dioxide (NO2) emitted by the burning of fossil fuels combines with atmospheric oxygen to yield O3 and nitric oxide (NO). Ozone is the major air pollutant affecting US crop yield. Ozone enters plants through microscopic pores in the leaf surface called stomata. Stomatal apertures and hence rates of O3 uptake into the plant are increased or decreased by swelling or shrinking of pairs of guard cells, which border and define the stomatal pore. We have shown that O3 inhibits the opening of K+-selective ion channels in the guard cell membrane. These channels are tiny proteinaceous conduits for K+ ion movement across the cell membrane. Inhibition of K+ uptake in turn inhibits water uptake by the guard cells, cell swelling and stomatal opening. Ozone was demonstrated to indeed inhibit stomatal opening in the intact leaf, as assayed by a technique known as gas exchange. In this project, we will assess whether O3 inhibits K+channels by directly oxidizing the channel molecule. We will also determine whether O3 affects another type of ion

channel in the guard cell membrane, the anion channel. Finally, we will assess whether there is an interaction between ozone and drought in inhibiting recovery of stomatal opening in intact plants following a drought episode. These experiments will provide new information on the mechanisms by which O3 alters guard cell function and plant productivity. This knowledge may be useful in the production of plants with improved O3 tolerance.

**2000-00649 Gordon Conference Cellular Basis of Adaptation to Salt and Water Stress in Plants**

Bray, E.A.

Gordon Research Conferences; University of Rhode Island; Kingston, RI 02881

Conference Grant; Grant 00-35100-9165; $10,000; 1 Year

The fourth Gordon Conference on the Cellular Basis of Adaptation to Salt and Water Stress in Plants will be held August 20-25, 2000 in Tilton, New Hampshire at the Tilton School. The Chair of the Conference is Elizabeth Bray (University of California, Riverside) and the Vice-Chair is P. Michael Hasegawa (Purdue University). The first Conference (held in August 1994) was focused solely on salinity. The second and third Conferences (held in August 1996 and 1998) were broadened to include cellular aspects of water stress and succeeded in attracting a wider range of researchers, bringing new ideas to both salt- and water-stress research, and ultimately increasing the potential impact of this Conference series on agriculture. The fourth conference will continue this tradition. Specific goals of the conference planned for 2000 are: (1) to highlight the latest progress in salt and water stress research at the cellular level, particularly in the fast-developing areas of ion transport proteins, ion and water channels, signal transduction, and the interaction with other stresses; (2) to integrate results obtained at the cellular level with whole plant responses in the agricultural context; (3) to consider how advances at the cellular and molecular levels can be applied in breeding or genetic engineering of plants for enhanced tolerance to salt and water stress; and (4) to bring together established and beginning researchers from North America, Latin America, Europe, Japan and Australia and to promote extensive formal and informal discussions on current research problems and future opportunities.

**2000-00491 Photosensory Receptors & Signal Transduction Gordon Conference, 2000**

Spudich, J.

Gordon Research Conferences; University of Rhode Island; Kingston, RI 02881

Conference Grant; Grant 00-35100-8969; $10,000; 1 Year

The Gordon Research Conference (GRC)-sponsored meeting on Photosensory Receptors & Signal Transduction is the first comprehensive conference organized around the theme in recent years and the first on this subject to be sponsored by the GRC. It was proposed in view of the recent breakthrough discoveries of new photosensory receptors and rapid progress on their activation and signaling mechanisms over the past 5 years. The focus is on molecular mechanisms of receptor phototransduction and brings together investigators of structure/function of microbial, plant, and animal photoreceptors. The conference will stimulate discussion of emerging insights, general principles, and current approaches on the cutting edge of photosensory signal transduction. This area of research is a fast-moving one, and the key objective is to discuss progress being made on several

fundamental questions: By what mechanisms is photon energy captured and stored in photoreceptor proteins? How is light color discriminated? How do photoactivated receptor proteins communicate their signals to the cell? How do organisms avoid light wavelengths and fluence rates that cause cell and tissue injury? The meeting will provide a very much-needed opportunity to exchange information and ideas and for investigators to become aware of the diverse and incisive molecular tools available.

**2000-01261 Environmental Effects on Xylem Cavitation**
Matzner, S.L.
Augustana College; Department of Biology; Sioux Falls, SD 57197
Seed Grant; Grant 00-35106-9403; $75,000; 2 Years

Global warming may decrease precipitation in many areas and reduce agricultural production. A better understanding of the mechanisms that determine drought tolerance may enable us to improve drought resistance in crops. Resistance to xylem cavitation is one important characteristic that determines drought tolerance. As drought stress increases in plants, tension within water-conducting cells (xylem) can aspirate (suck in) a bubble (cavitation). This reduces water transport, resulting in the closure of stomata, which reduces photosynthesis and growth. Resistance to cavitation is determined by the diameter of inter-xylem cell pores, which determine the tension at which a bubble is aspirated. Selecting crops for small pore size would increase drought tolerance but unfortunately smaller pores may be correlated with smaller xylem cell size. Smaller xylem cells also mean less water transport capacity, stomatal closure, and reduced growth. However, if pore size can vary independently from xylem cell size, it may be possible to have both large xylem cells and small pores. A plant could therefore, have both a high potential for growth, but also be drought tolerant. This study will vary environmental factors to alter xylem and inter-xylem cell pore dimensions to determine if they can in fact vary independently of each other. This research will contribute to our understanding of the fundamental mechanisms that determine resistance to xylem cavitation and how they may be altered by the environment. Understanding these relationships may enable us to predict and perhaps even manipulate the drought tolerance of plant species in the face of looming climate change.

The objectives of this study are to better understand the mechanisms that determine drought tolerance in crop species. Specifically this study will focus on xylem cavitation and whether pore size can vary independently from xylem cell size, making it possible to have plants with both a high potential for growth, but are also drought tolerant. This proposal will use *Phaseolus vulgaris* (common bean) as a model species to address: (1) How various environmental factors (nutrients, soil moisture, humidity and temperature) affect xylem cavitation, xylem cell size and inter-xylem cell pore size; and (2) If alterations in xylem-cell pore size is independent of changes in xylem cell size or is correlated with xylem cell size. The answers to these questions will be sought using recently developed techniques for determining cavitation resistance on herbaceous species and through measurements of xylem cell and inter-xylem cell pore size and structure.

**2000-00650 Mycorrhizal Soils and Plant Drought Resistance**
Augé R.M.; Sylvia D.M.

University of Tennessee, Knoxville; Institute of Agriculture; PO Box 1071; Knoxville, TN 37996-4500.
Grant 00-35100-9238; $100,000; 3 Years.

Mycorrhizal symbiosis is a naturally-occurring partnership between plant roots and certain soil fungi, often having remarkably positive and vital consequences for agriculture, forestry and native ecosystems. The symbiosis is a characteristic of healthy soils, both native and cultivated, that helps plants better absorb nutrients, fight pathogens and withstand environmental stresses. Mycorrhizal symbiosis also protects and enhances soil tilth, improving structure and resilience to erosion. Some modern agricultural practices have diminished these beneficial fungi in soils, but scientists are learning how to re-establish the symbiosis. Our project will investigate the impacts of various mycorrhizal fungal species and species combinations on soil aggregation and on the associated proficiency of soils for retaining moisture. We will relate this information to the ability of mycorrhizal fungi to allow crops to maintain more normal (closer to "non-stress") behavior during drought. Mycorrhizal symbiosis has been implicated in increased crop drought resistance. We will determine whether some or all of the mycorrhizal benefit is tied to mycorrhizal changes in soil water retention properties. By studying both the soil and plant components of the mycorrhizal system in tandem, our project will provide an in-depth characterization of how re-instating and encouraging this natural soil symbiosis protects both soils and plants from environmental extremes.

**2000-00646 Physiology and Molecular Regulation of Zinc Homeostasis in *Medicago truncatula***

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Grant 00-35100-9365; $142,000; 3 Years

Zinc (Zn) is an element essential for plant life but which also is toxic in high amounts. Plants must carefully regulate its uptake in order to ensure adequate intake while preventing excess accumulation. Our present knowledge in Zn nutrition is focused primarily on Zn transport phenomena, with recent efforts having led to the identification of a number of Zn transport proteins. Unfortunately, we know little about how these transporters are functionally integrated at the whole-plant level to ensure adequate and proper Zn nutrition. In this project, we will study Zn nutrition in a unique mutant of the model legume, *Medicago truncatula*. This novel mutant is functionally Zn deficient; it exhibits symptoms of Zn deficiency in leaves, even though it also overaccumulates Zn. We hypothesize that Zn is being inappropriately utilized in this mutant, such that inadequate levels of Zn are available and/or are being sensed within the plant. We propose that the mutant responds to this functional deficiency by upregulating Zn transport mechanisms. We will exploit the attributes of this mutant to characterize Zn availability throughout the plant, to determine zinc uptake kinetics in roots and leaves, and to identify and charcterize zinc transporter genes in *Medicago truncatula*. The results of these objectives will provide a clearer understanding of the molecular components and processes that govern Zn nutrition in higher plants and will yield strategies for how Zn uptake could be manipulated to benefit plants in both limiting and toxic environments.